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Planthopper “adaptation” to resistant rice varieties: Changes in amino acid composition over time

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ABSTRACT

The brown planthopper, *Nilaparvata lugens*, shows considerable geographic and temporal variability in its response to varieties of cultivated rice. *N. lugens* has repeatedly “adapted” to resistant rice varieties; however, the physiological changes underlying planthopper adaptation are poorly understood. Endosymbionts within planthoppers, such as yeast-like endosymbionts (YLS) could play a role as they produce essential amino acids for planthoppers. We used a full factorial study to determine how natal rice variety, exposed rice variety, YLS presence, and the number of reared generations affected nymphal development, planthopper total nitrogen content, and planthopper hydrolyzed amino acid profiles. Nymphal development was strongly influenced by a four-way interaction between the exposed rice variety, natal rice variety, number of reared generations, and YLS presence. While symbiosis improved nymphal performance in the 8th generation, it appeared to be a drain on nymphs in the 11th generation, when the aposymbiotic nymphs actually showed higher performance than the symbiotic nymphs. This suggests that the symbiotic relationship may be acting beneficially in one generation while acting as a drain during another generation. Aposymbiotic planthoppers reared for 11 generations had a higher proportional concentration of rare amino acids than those reared for 8 generations, indicating that the planthopper itself appears to improve its ability to acquire rare amino acids. Therefore, the change in amino acid composition of planthoppers suggests an underlying change in protein expression or amino acid metabolism over time.

1. Introduction

The brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae), shows considerable geographic and temporal variability in its response to cultivated rice varieties (Heinrichs et al., 1985; Pathak and Khush, 1979; Pathak and Heinrichs, 1982). As a product of the Green Revolution (Kenmore et al., 1984), *N. lugens* outbreaks have threatened rice production and food security (Cohen et al., 1997; Dyck and Thomas, 1979). Although there has been significant investment in breeding for rice resistance to the brown planthopper in Asia (Cha et al., 2008; Cohen et al., 1997; Heinrichs et al., 1985; Jairin et al., 2007; Jena et al., 2006; Lu et al., 2007; Park et al., 2007; Su et al., 2006; Sun et al., 2007), the emphasis on gene discovery for planthopper resistance has severe shortcomings. *N. lugens* has repeatedly shown the ability to rapidly “adapt” to resistant rice varieties after several generations of continuous rearing in the field and laboratory (Claridge and den Hollander, 1983; Claridge et al., 1982, 1984; Gallagher et al., 1994). We use the term “adaptation” to describe how planthoppers increase their performance on a single rice variety over several generations. Given that planthopper “adaptation” occurs over months, it appears to occur too rapidly to result from selection. Before lasting progress can be made in breeding for rice resistance, it is important to determine what factors underlie planthopper “adaptation” to resistant rice varieties.

Planthopper “adaptation” to resistant varieties can be measured through increases in survival, body weight, honeydew production, and/or reproductive fitness (Pathak and Heinrichs, 1982). Host plant nutrition is thought to be more important than defenses, because delphacid planthoppers almost exclusively feed on monocots, which contain lower levels of plant allelochemicals (Harbone and Williams, 1976; Prestidge and McNeill, 1983). Resistant rice varieties generally have higher levels of phenolic compounds, lower levels of free amino acids, and lower levels of reducing sugars (Das, 1976; Grayer et al., 1994; Mishra et al., 1990; Thayumanavan et al., 1990). Variation in amino acid abundance and composition may affect planthopper fitness and devel-

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opment as well. On nitrogen-deficient rice plants, *N. lugens* switch their feeding sites more often, and as a result, excrete less honeydew (Sogawa, 1982). Also, varieties with lower asparagine content are considered to be resistant to planthoppers (Chino et al., 1987; Sogawa and Pathak, 1970).

Microbial symbiosis has been linked with insect groups that specialize in feeding on plant phloem (Douglas, 1998). These symbiotic microorganisms improve diet quality by synthesizing essential amino acids lacking in the insect's diet (Douglas, 1989, 1998). In this study, we denote "essential" amino acids as those amino acids found in planthoppers, but rare in the planthopper's diet, which consists mostly of the amino acids asparagine/aspartate and glutamine/glutamate. While aphid symbiosis has been studied in great detail, much less is known about planthopper endosymbionts and their role in planthopper metabolism (Wilkinson and Ishikawa, 2001). In planthoppers, yeast-like endosymbionts (YLS) reside intracellularly in the planthopper's fat body cells (Buchner, 1965; Cheng and Hou, 1996; Noda, 1974). YLS provide rare nutrients to the planthoppers to compensate for the unbalanced composition of amino acids in plant phloem (Noda and Saito, 1977, 1979). When YLS are experimentally removed, *N. lugens* nymphs lose weight and grow more slowly (Wilkinson and Ishikawa, 2001). Without YLS, planthoppers have lower total protein concentrations, higher levels of non-limiting free amino acids such as glutamine and aspartate, and significantly lower levels of other amino acids such as leucine (Wilkinson and Ishikawa, 2001). It is difficult to speculate on how reliant *N. lugens* are on YLS because artificial rearing experiments have shown that no single amino acid is considered limiting for *N. lugens* (Koyama, 1985). Experimental results confirm that YLS play a role in amino acid metabolism through the recycling of uric acid (Sasaki et al., 1996). Therefore, YLS appear to support both planthopper nutrition and development.

N. lugens reared continuously in culture appear to physiologically specialize on the rice variety that they are reared upon within several generations, but show a reduced performance on other varieties (Claridge and den Hollander, 1980; Claridge and Den Hollander, 1982; Saxena and Barrion, 1983). These selected populations have been named "biotypes" in other studies, but the use of this term is problematic. The rapid "adaptation" of *N. lugens* on reared rice varieties strongly suggests that the "biotypes" appear to be selected populations rather than genetically distinct and diverging host races (Claridge and den Hollander, 1983; Shufran and Whalon, 1995). However, other biological factors could contribute to these patterns of "adaptation". When transferred from a susceptible rice variety to resistant rice varieties (ASD7 or Mudgo), Lu et al. (2004) found that planthoppers showed a decrease in nymphal performance and survival in the first generation, coupled with a decrease in YLS densities and transaminase activity. In the subsequent 2nd and 3rd generations, nymphal performance improved and YLS became more abundant. As a result, Lu et al. (2004) suggested that YLS may be linked to variation in planthopper performance and adaptation to rice resistance.

In this study, we examined if planthopper amino acid composition and development was influenced by the natal rice variety, the exposed plant variety, YLS presence, or the number of generations associated with a particular variety. We used a full factorial design to assess the relationship between the number of generations reared on a host plant, presence of YLS, natal plant variety, and exposed plant variety on planthopper amino acid composition and performance. We assessed planthopper nutrition by examining planthopper total nitrogen content and hydrolyzed amino acid profiles. Specifically, we used the full factorial design to ask: (1) How does continuous exposure on one rice variety lead to trade-offs in performance and amino acid composition (nymphal development, planthopper total nitrogen content, and proportional levels of hydrolyzed amino acids) on another rice variety? (2) How does the presence of YLS influence planthopper metabolic trade-offs on different rice varieties? 3) How does the natal host plant, exposed host plant, number of generations interact to influence planthopper amino acid composition and performance?

2. Methods

2.1. Rearing of study organisms

We used three rice varieties Taichung Native 1 (TN1), Mudgo, and ASD7 that vary in their resistance and have been extensively used in studies of planthopper adaptation to resistant rice varieties (Claridge and den Hollander, 1980; Claridge and Den Hollander, 1982; Saxena and Barrion, 1983; Saxena and Pathak, 1979). TN1 is considered to be susceptible to *N. lugens*, while Mudgo is thought to carry the *Bph 1* gene for resistance and ASD7 is thought to carry *Bph 2* for resistance (Heinrichs et al., 1985). Susceptible varieties tend to have higher levels of free amino acids than resistant rice varieties (Sogawa, 1982; Thayumanavan et al., 1990). The rice varieties chosen for this study also significantly differ in amino acid content, with Mudgo and ASD7 showing lower levels of free amino acids than susceptible varieties such as TN1 (Saxena, 1986; Sogawa and Pathak, 1970).

A colony of *N. lugens* has been continuously reared in the greenhouse since the early 1960's on the susceptible variety, Taichung Native 1 (TN1), at the International Rice Research Institute (IRRI) in the Philippines. Since 2004, field-collected planthoppers have been added annually to prevent excessive inbreeding. Using the TN1 colony, we establish three colonies on the rice varieties TN1, Mudgo, and ASD7 using an initial population of 500 planthopper adults. Planthoppers were reared on TN1, Mudgo, and ASD7 for 5 generations as three separate colonies before the start of the experiment.

TN1, Mudgo, and ASD7 plants were raised by sowing seeds into plastic trays (51 × 39 × 10 cm) half-filled with field-collected top soil. Seven days after sowing, a single rice seedling was transplanted into a circular clay pot (7 cm diameter × 12 cm depth). Plants were fertilized at a rate consistent with field practices (150-60-20 NPK). The pots were held in galvanized iron trays filled with water to simulate flooded conditions. The plants were reared for 10 days in the greenhouse before use in the study.

2.2. Treatments

We raised plants in the glasshouse at the International Rice Research Institute. The experimental study was conducted in growth chambers (Thermo Scientific) at 25 °C under a 12:12 L:D light regime. We used a full factorial design to assess the role of the natal rice variety, exposed rice variety, YLS presence, and the number of reared generations on nymphal development, total nitrogen within the planthopper, and hydrolyzed amino acid profiles of the planthopper. The treatments consisted of three natal rice varieties (TN1, Mudgo, and ASD7), three exposed rice varieties (TN1, Mudgo, and ASD7), symbiosis (planthoppers with normal and reduced YLS abundance), and the number of generations (6th and 8th generation). The term "natal rice variety" refers to the planthopper culture that was started from a TN1 planthopper colony and reared on a particular variety throughout the duration of the study. For each assayed generation, planthoppers were taken each planthopper colony and exposed to one of the three rice varieties. Therefore, the full factorial study consisted of a total of 36 treatment combinations.

While the yeast-like endosymbionts cannot be completely eliminated like aphid bacterial endosymbionts, exposure to high temperatures can largely remove the endosymbionts without